Operational Report of Rat Eradication on Lehua Island

February 2009
Peter Dunlevy

United States Department of Agriculture
Animal and Plant Health Inspection Service
WILDLIFE SERVICES
Hawaii/ Guam/ Pacific Islands

1 Operational Summary

Title of report:	Operational Report of Rat Eradication on Lehua Island
Project Manager:	Peter Dunlevy
Objective:	Recovery and restoration of native species and ecology on Lehua Island via removal of invasive species
Target species:	Polynesian rat (*Rattus exulans*)
Treatment area:	Lehua Island surface area, ≈126 ha (312 ac)
Rodenticide formulation:	Diphacinone—50 (EPA Reg. No. 56228-35) aka Ramik® Green, fish flavored weather resistant 13 mm cereal pellets containing 50 ppm diphacinone
Application method:	Aerial broadcast
Application rate:	14 kg/ha (12.5 lb/ac)
Treatment dates:	First broadcast 6 Jan 2009, second broadcast 13 Jan 2009
Contractors:	Volcano Helicopters – Hilo, HI Inter-Island Helicopters – Port Allen, HI Pacific Helicopters – Kahului, HI American Marine – Honolulu, HI
Results:	Yet to be confirmed, efficacy monitoring is scheduled to take place in late summer of 2009 and 2010, 2 annual breeding cycles after the operation

2 Objectives

Eradication

- Complete removal of Polynesian rats from Lehua Island.

Recovery and restoration

- Increase in numbers of native plant species including: (*Scaevola taccada*, *Cyperus polystachyos*, *Eragrostis variabilis*, *Canavalia pubescens* and *Lepturus repens*)
- Increase in numbers of native invertebrate species including: *Hylaeus* bees, *Nysius* seed bugs and other native species still present.
- Increase in numbers of native seabird species including: masked booby (*Sula dactylatra*), brown noddy (*Anous stolidus*), blue-gray noddy (*Procelsterna cerulean*), Bonin petrel (*Pterodroma hypoleuca*), sooty tern (*Onychoprion fuscatus*) and gray-backed tern (*Sterna lunata*).
- Establishment of endangered native plant species including: *Pritchardia Aylmer-robinsonii*, *Cyperus trachysanthos* and *Brighamia insignis*.
- Establishment of native invertebrate species including: *Pleuropoma niihauensis*, *Lamellidea gracilis*, *Agrotis dislocata*, *Aloha myoporicola* and *Asyendetus carcinophilus*.
- Establishment of endangered bird species including: Hawaiian petrel (*Pterodroma sandwichensis*), Newell's shearwater (*Puffinus auricularis newelli*), band-rumped storm petrel (*Oceanodroma castro*) and Nihoa finch (*Telespiza ultima*).
- Recovery of ecosystem processes and function, including vegetation, invertebrate and avian communities.

3 Operational details

Lehua Island is situated approximately 240 km (149 mi) north northwest of Honolulu and 1125 m (0.7 mi) north of the northern most point of Niihau at 22 1' North latitude 160 05' West longitude.

Native bird species that were potentially present during the operation included: **Laysan albatross** (*Phoebastria immutabilis*), **black-footed albatross** (*Phoebastria nigripes*), **wedge-tailed shearwater** (*Puffinus pacificus*), **white-tailed tropicbird** (*Phaethon lepturus*), **red-tailed tropicbird** (*Phaethon rubricauda*), **red-footed booby** (*Sula sula*), **brown booby** (*Sula leucogaster*), **great frigatebird** (*Fregatta minor*), **black noddy** (*Anous minutus*). Introduced bird species that were potentially present during the operation: **common barn owl** (*Tyto alba*), **cattle egret** (*Bubulcus ibis*), northern cardinal (*Cardinalis cardinalis*), house finch (*Carpodacus mexicanus*) and nutmeg manikin (*Lonchura punctulata*).
*Bolded species were observed during operations

Although Lehua is currently dominated by non-native plant species, the existence of a once present native dry forest on Lehua indicates a different past. Lehua has 23 native plant species remaining in very low numbers, several of which are rare and vulnerable. Like the plants, Lehua's terrestrial arthropod community is dominated by non-native species, including ants and grasshoppers, although endemic *Hylaeus* bees, *Nysius* seed bugs and a few other native species are still present. Recent surveys documented over 25,000 breeding pairs of seabirds and up to 11 species nesting or attempting to nest on Lehua. Wedge-tailed shearwaters are the most numerous species on the island, but Lehua has the largest brown booby colony and one of the two largest red-footed booby colonies in Hawaii. Lehua is the only known nesting location in the main Hawaiian Islands for the rare black-footed albatross, which were first documented nesting on Lehua in 2001. Laysan albatross, another species rarely seen in the main Hawaiian Islands, also nest on Lehua. Band-rumped storm petrels, threatened Newell's shearwaters and endangered Hawaiian petrels have also been observed in the area of Lehua.

Newell's shearwater, Hawaiian petrel, Hawaiian monk seal (*Monachus schauinslandi*), and green sea turtle (*Chelonia mydas*) are ESA listed species either present or potentially present on Lehua.

Weedy plant species, such as lantana, pluchea, christmasberry and others pose a threat to native plants by competing for habitat.

The flora consists of 49 species of which 22 are native. The vegetation of Lehua is predominately a *Cenchrus ciliaris-Setaria verticillata-Portulaca oleracea-Jacquemontia ovalifolia* association interspersed with other common associates including the dominant shrubs *Pluchea indica*, *Pluchea carolinensis*, in addition to other herbaceous species such as *Ageratum conyzoides*, *Chenopodium murale*, *Waltheria indica*, *Cenchrus echinatus*, and *Chloris virgata*.

Mean annual rainfall is 733 mm, which is highly seasonal, ranging from 15 mm per month in December to 148 mm in March. It rains more than 12 mm on between 0 and 4 days a month. Mean temperatures range from 22˚ C in February to 26˚ C in September. Trade winds are fairly uniform throughout the year, however, there are more frequent high winds in the winter months with occasional gusts over 30 mph and infrequent gusts over 40 mph. North Pacific winter storms produce potentially large ocean swells from that direction.
There are no inhabitants; however, there is fishing in many areas along the shore of Lehua. Fishing is primarily during the summer since winter seas are often very rough. Interviews with several subsistence users in December 2007 indicated that fishing near Lehua is primarily for non-resident, pelagic species.

Historical use of the island is unknown and although rock structures are present on Lehua, the nature and source of these rock structures are unidentified. The difficulty of accessing Lehua and the steep rugged topography combined with the lack of water and soil make it unlikely that it was inhabited in the past.

Rabbits were targeted for eradication in 2005-06 using mechanical means, invasive barn owls and cattle egrets are periodically controlled and an attempt at mechanical eradication of *Verbesina* is ongoing. Basic vegetation and avian monitoring has been conducted (Wood et al 2003).

4 Notification, Consultation and Public Relations

Public notification was made in the State of Hawaii Office of Environmental Quality Control (OEQC) Bulletin, published on 12 October 2008. Seven written comments were received in response to public notification, none opposed. Informal discussions and public meetings were held on Kauai 24 July 2008. Kauai residents were supportive of the concept of conserving native species through rat eradication. However, concerns about the project were reflected in the questions that were asked about marine testing, possible impacts to birds, bait application logistics, and breakdown of bait pellets. These questions were answered in detail with the information already contained within the draft and final EAs.

Consultation took place with Kauai residents, tour operators, fishermen, members of the Niʻihau community living on Kauai and kupuna.

PR was proactive in the local community and began long before the project. Information was provided to the community regarding diphacinone, its safe record of use in agriculture and commensal rodent control, and research conducted in Hawaii and elsewhere on environmental fate and the risk of nontarget hazards.

5 Regulation

- The State of Hawaii Pesticide Branch was the local authority and provided regulatory approval for aerial broadcast of Diphacinone—50 via permit K08-01 to apply a restricted use pesticide by aircraft issued on 1 December 2008.
- National Environmental Policy Act (NEPA) – Federal and State, EA and FONSI issued 30 September 2005, Final Supplemental EA and FONSI issued 10 October 2008
- Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) – Fed, §3 label approved by EPA 9 December 2007
- Hawaii Pesticide Code – State, §3 label accepted by State Pesticide Branch December 2007, Applicator certifications, Pesticide dealer's licence issued by HDoA
- Endangered Species Act (ESA) – Federal (addressed in EA)
- Marine Mammal Protection Act (MMPA) – Federal (addressed in EA)
- Migratory Bird Treaty Act (MBTA) – Federal (addressed in EA)
- Coastal Zone Management Act (CZMA) – State (addressed in EA)
- Clean Water Act (CWA) – Federal and State (addressed in EA)
- Fishery Conservation and Management Act (FCMA) and Essential Fish Habitat (EFH) - Federal (addressed in EA)
- Hawaiʻi State Wildlife Sanctuary – State (addressed in EA)
- Hawaiʻi Comprehensive Wildlife Conservation Plan - State (addressed in EA)
- National Historic Preservation Act (NHPA) – Federal and State (addressed in EA)
- Subsistence Uses – Federal and State (addressed in EA)
- Kauai Conservation Plan and Policies - County (addressed in EA)

Mitigation measures incorporated into operation:

- Ground crews conducting monitoring or other restoration activities on Lehua will maintain a 100 foot distance from Hawaiian monk seals hauled out on the shoreline
- The helicopter will be required to avoid flying over or spreading bait onto any Hawaiian Monk Seals hauled out on Lehua
- The helicopter will be required to avoid flying over Humpback Whales
- No vessel associated with the project will approach within 100 yards of humpback whales
- The helicopter will fly over land when distributing bait pellets
- Diphacinone, a highly effective yet far less toxic rodenticide than that most often used for rat eradication, will be the first choice for use on Lehua.
- The helicopter pilot will guide and record bait application with an on-board differential global positioning system (GPS), assuring uniform and complete coverage of the island without over-application
- To avoid bait being washed into the ocean by rain before it is consumed by rats, bait will only be applied when no significant rainfall is forecasted
- To avoid uncontrolled bait spread and to comply with pesticide label requirements; bait will not be applied when winds exceed 35 mph (30 knots)
- Marine monitoring will be conducted following bait application and near-shore samples of water, fish, and invertebrates will be tested for rodenticide residues. Test results will immediately be made available to agencies and the public.

6 Operations

The time dedicated to project planning and management proved sufficient to organize the project. However, it always takes more time than one thinks when coordinating other people, especially those with other jobs to do, and as was shown on a couple of occasions if the PM doesn't follow objectives all the way through things can go wrong. It would also have been beneficial to have an assistant for the PM for 2-3 months prior to the operation. This person could have taken responsibility for some of the tasks leaving the PM to focus on others and the overall picture. Also, should the PM not be able to follow thru with the project for any reason a ready stand-in is available. The use of standard operating procedures (SOPs) during projects such as this over the long term is vital and should be developed - a list of all the relevant SOPs should be compiled for future planning (e.g. calibration, bait loading, bait monitoring, nontarget carcass search, etc.).

An oversight/ advisory panel in the US, which is in tune with the situation and issues here, would greatly improve preparation and coordination of similar projects. If a qualified panel can be established for future operations in addition to planning and technical advice I suggest that there be two reviews: one approximately 4 months prior to the operation to ensure that preparation is on track and giving the PM plenty of time to conduct any resulting work and one about 3-4 weeks out to ensure that all necessary preparation has been done.

Many team members were located on various islands and could not be brought together without considerable expense and effort, therefore the full team was not briefed in person prior to departure. Briefings were held with monitoring team leaders prior to departure for Lehua. These briefings focused on the tasks required from the team prior to getting everybody together on the site and included safety. Once the field team was assembled on site discussions were held covering task responsibilities, safety etc. as needed.

A periodic update for key project participants and team members to keep them informed of progress and what they should be doing could be used in future projects. As well as what they would be required to do etc., the team were also given copies of all the plans - operational, safety etc, to comment on so that they were aware of the "big picture". Possibly a web page could be developed to keep both staff and other interested people updated on the project's progress.

There were no accidents during the field project. Safety was reinforced at every briefing with any concerns raised.

The field team ended up consisting, at times, of a total of 15 people including three pilots. This proved adequate, although some project tasks were not able to be finished in the desired time period. In addition, on many occasions the project manager was required to play a more hands on part in the lead up to the operation than was desirable, which reduced his ability to coordinate the whole operation (e.g., maximise resources/ safety etc.). For example, the PM had to personally prepare all field gear and arrange shipping as well as physically transport the gear to and from shippers during the final critical days in the lead up to the operation when other overall coordination and last minute preparations needed to be made. Staffing was obtained ad hoc from cooperators and interested parties who did not

fully understand the time commitment, training and coordination required. As a result, the necessary time required of these volunteers was not fully supported. Nine of the 15-person field team were volunteers from the FWS or State agencies who had many other responsibilities and did not formally work on or get paid by the project. Only two field crew, besides the PM and the pilot, had any previous rodent eradication experience. Involving WS staff in the operation that are to work on future operations is important for knowledge sharing, it is also important to have WS staff who have worked in previous operations for the same reason. The application pilot - David Okita, gear transport pilot - Ken D'Attilio and crew transport pilot – Lawrence Guillermo were excellent and very skilled.

A MD500E helicopter with a custom made agricultural broadcast spreader suspended beneath was used to apply the rodenticide. No directional curtain was installed. This would have theoretically reduced the amount of bait going to one side and increased the amount of bait going onto the shoreline. While a good potential idea, the design was susceptible to pellets bouncing off the curtain and into the belts causing them to slip off the pulleys and requiring many repairs. In addition, testing has shown the deflector does not work: some bait is pulverized to dust, still goes to the 'unwanted' side and accumulates at very high rates directly below. Engineers are following up on this idea and may have a directional design ready for future use. This concept could be further explored for future operations. A Trimble® Trimflight 3 DGPS was used to ensure accuracy and document the broadcast.

20,000 lbs (9,072 kg) of Diphacinone-50 rodenticide were ordered from Neogen Corporation's Hacco factory (discussion began in August and final order confirmed 9/26/2008) thru the WS Pocatello Supply Depot. This was delivered in a 20 ft container to Honolulu where it was stored. Post operation, three random samples of bait were sent to the NWRC chemistry lab for diphacinone concentration verification.

Six and one-half loads of bait or 3,900 lbs were required for each broadcast of the treatment. Project personnel loaded bait using mechanical bulk loading procedures. Brailer bags, each holding one full spreader load, were used to transfer bait to the spreader. After the pilot/ helicopter placed the empty spreader in the loading area and hovered off to the side the pilot gave the go ahead for loading. Bulk bags were lifted over the empty spreader via machine (forklift) and the bait released into the ag-spreader below.

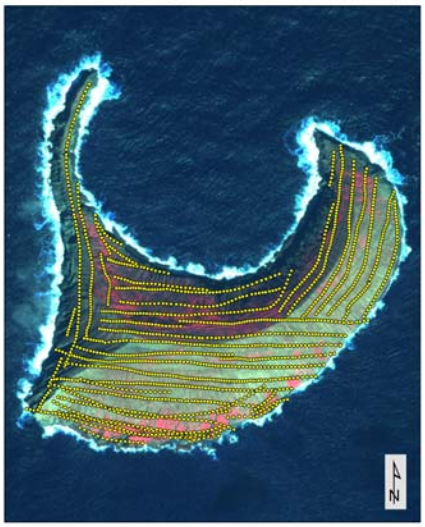
The weather during the operation was crucial. We were fortunate to get sufficient good weather when necessary. Weather forecasts were delivered via the National Weather Service and a weather station installed on Lehua for the operation. This gave a daily forecast for up to 7 days. The most important part of the forecast was the rain prediction, as a heavy rain (>13 mm) forecast during the next three days disallowed bait application even if conditions allowed for it on that day. The forecasts were as accurate as could have been expected and local knowledge by the PM and team members meant that no unrealistic expectations were made. Winds were light, 10-15 mph and from the NW during both broadcasts. There was very little rainfall in the month following broadcasts. The only significant rain event in the month after broadcast was approximately 0.7 inches on January 11th.

The team arrived on Lehua 29 December and began setting up for monitoring broadcast operations thru 3 January. Baseline water, soil, limpet, crab and fish samples were collected; a pre-operation nontarget carcass search and an extensive trapping effort to radio collar rats were conducted. While trapping was not actively used as an eradication method, it was used for specific tasks. Trapping was used to confirm rat presence and annual population parameters prior to the operation and will be used to ensure that all rats were removed. The catch rate just prior to the operation was very low, similar to earlier assessments at that season (Dunlevy 2007). Only one rat was captured during 583 trap nights between 29 Dec and 10 Jan, as the operation was timed to coincide with the target species annual abundance and breeding low, therefore telemetry was impossible.

5 January – notification made to HDoA, the barge loaded with broadcast materials in Honolulu and all participants advised that the operation was to proceed the next day.

6 January – the first broadcast application, GIS assessment, bait monitoring and albatross mitigation.

Lehua Island Bait Drop - Helicopter Transects (1-06-09)



7 January – 24-hr marine monitoring samples taken (water, soil, limpet, crab and fish) and bait monitoring data collected.

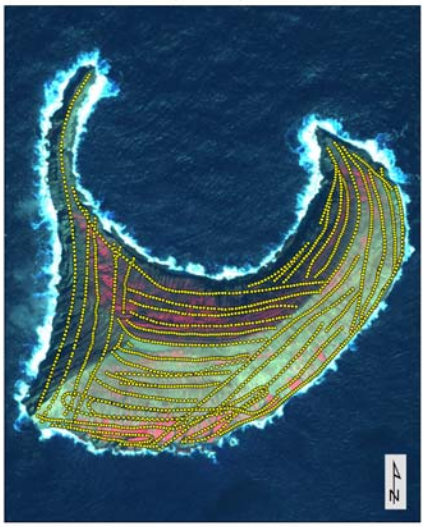
10 January – bait monitoring data collected, nontarget search conducted and the barge loaded with broadcast materials in Honolulu.

11 January – notification made to HDoA and all participants advised that the operation was to proceed the next day.

12 January – barge did not depart HNL as requested, all other assets called off and broadcast postponed until the following day.

13 January – the second broadcast application, GIS assessment, bait monitoring, albatross mitigation and 24-hr/ 7-day marine monitoring samples taken (water, soil, limpet, crab and fish).

Lehua Island Bait Drop - Helicopter Transects (1-13-09)

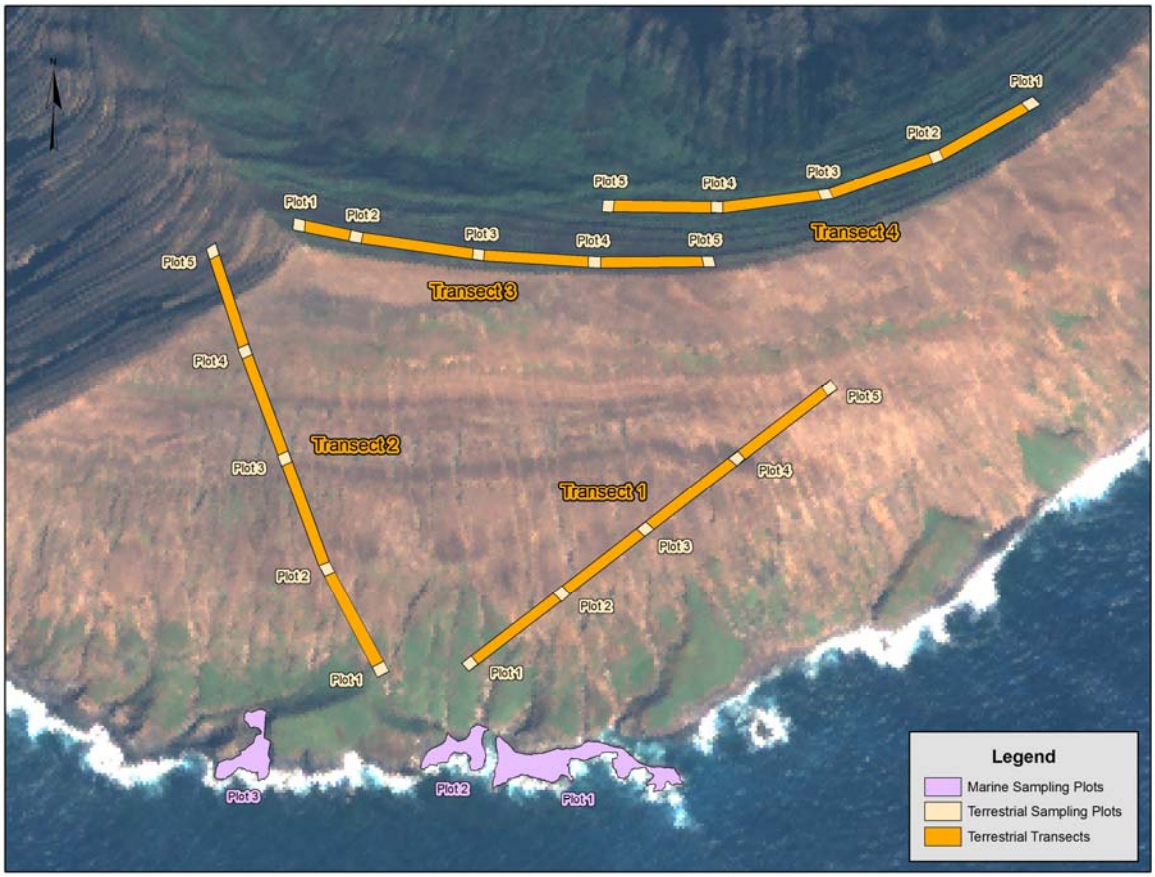


17-19 January – bait monitoring, albatross mitigation, 7-day marine monitoring samples taken (water, soil, limpet, crab and fish) and nontarget search conducted.

20-21 January – breakdown and return gear to Honolulu.

7 Bait Application Monitoring

To ensure the bait was broadcast uniformly over the entire island, we used a Trimble AgGPS Trimflight 3 System. The AgGPS Trimflight 3 System was used to accurately map the island boundary and record every flight path for each application event. All data was stored in ESRI shapefile format on a removable compact flash (CF) card. The AgGPS Trimflight 3 System in-cockpit display and lightbar provided the pilot instantaneous guidance along flight lines. Global Positioning System data was downloaded from the CF card during bait reloading and helicopter refueling events. Once downloaded, GIS and GPS data were imported into ArcGIS to track and document bait distribution. On-the-ground measurement also assessed the rodenticide application and records of the total amount of bait applied and the treatment area were kept. The number and weight of pellets within 20 census plots were recorded immediately after each broadcast as an evaluation of the bait distribution. Notes regarding substrate and slope were documented during this appraisal. After rodenticide application a sample of pellets were located within plots and marked with survey flags. Marked pellets were monitored throughout treatment until they disappeared, biodegraded or for 14 days. The light rain that occurred overnight on the 10-11th did not significantly affect bait distribution. Plots with exposed rock surface and greater than 30-degree slope had some pellets migrate downhill less than 10 m.



8 Nontarget and Environmental Monitoring

Diphacinone does not affect invertebrates, seabirds do not eat on land, and the break-up and dilution of any small amount of bait that may have bounced or rolled into the sea is so great there were not likely to have been any effects on the ecosystem, much less significant effects.

There was a slight potential of some introduced passerine or owl exposure and the public perception of rodenticide entering the marine food chain was ever present. These were monitored during the operation. No nontarget mortality was documented during formal searches or incidentally throughout the month-long monitoring efforts. No diphacinone residues were detected in any samples taken of three taxa monitored across three sampling periods from

7 thru 20 January. Toxicant choice, highly accurate broadcast application and operation timing eliminated these problems.

On 2 February dead fish were reported to be washed up primarily at a beach completely around the other side of Niʻihau from Lehua, 17 miles away (straight line distance over Niʻihau) and soon after on 9 February a whale calf washed up on Kauai. A State (DAR/ DoH) investigation ensued, including a pesticide use/ misuse inspection (HDoA) of the Lehua Project. Results of Niʻihau fish testing coordinated by State officials indicated deaths attributed to a biotoxin — cyanobacteria, or blue-green algae — which was found in the stomachs of specimens collected February on Niʻihau. Ephemeral lakes on Niʻihau might have had cyanobacteria that could have been discharged during the last week of December when Niʻihau experienced a major winter storm and heavy run-off. It is the only link to a toxin that could have caused the fish kill. No diphacinone residues were detected in any fish, seals or the whale. Necropsies and testing revealed no pathology linking any of these mortalities with diphacinone toxicosis. Water and Soil were monitored during the operation and no diphacinone residues were found. (see appendix A)

9 Efficacy Monitoring

To be conducted starting August 2009. This will include standardized trapping as conducted prior to the operation. Accepted practice to confirm the success of rat eradication is to wait for 2 years and then carry out monitoring using traps or other devices.

Monitoring Dates: August 2009 and 2010.

10 Ecological Monitoring

Plants, invertebrates and birds will be monitored using the same techniques used for the base line monitoring on an opportunistic basis.

11 Budget

Unlike most projects where a plan is made and the cost estimated to give a budget, the budget for this operation was arrived at ahead of the operational planning. There were so many unknowns in the project, e.g. how long it would take/ standby, etc. that an accurate budget was impossible. If everything had tended toward the worst case scenario, money would have been an issue (e.g. trying to calculate the exact amount of money for helicopter time while leaving money for monitoring, shipping, travel, etc.). It is important to remember that as shown by previous operations things always cost more than you expect. The management of the budget was primarily carried out by the FWS, the funding agency. As there are still some expenses required, the final total will not be known for a couple of months. In addition, not all costs associated with the project have been incorporated, including some major categories such as salaries.

Basic Costs

Description	Cost
Equipment	$23,000
Bait	$25,750
DGPS/ GIS	$10,050
Helicopter	$72,000
Barge	$111,000
Monitoring	$42,500
Planning/ Regulatory	$35,500
Outreach	$8,500
Travel	$20,000
Shipping/ Storage	$5,000
Overhead	$18,000
Totals	$371,300.00

12 Media

Media coverage was adequate before the operation. It would be very useful to have thorough media releases and information sheets prepared well before, along with relevant photos and if possible video footage. Also, a media plan for addressing problems that arise should be considered.

13 Recommendations

- Get experienced staff to commit to the required time frame early. Understaffing at critical times undermines the project. Include experienced past technicians and inexperienced future technicians to maintain and build capacity.
- Assistant PM for 3-4 months prior to the operation.
- Detailed discussions with regulatory agencies and the necessary approvals should be agreed upon and applied for well in advance to allow time for any unexpected delays and remain in force for multiple years in case of unexpected delays.
- Discussion should be proactive and take place well in advance of the operation, which would reduce objections to obtaining approvals or problems with carrying out the operation.
- Media strategy in place prior to operation to quickly address any problems that arise.
- SOPs developed and compiled for future eradication project managers.
- Oversight panel carry out review of future projects.

14 Future Management

The highest priority for management of Lehua Island is quarantine. This covers not only rodents and other invasive vertebrates, but also invertebrates and weeds. This will be managed under the OIRC quarantine plan and requires adequate resources to be carried out effectively.

15 Appendices

Monitoring reports: Environmental Fate (soil and water) and Nontarget (terrestrial and marine)

Wildlife Services NWRC National Wildlife Research Center Analytical Services Report	United States Department of Agriculture Animal Plant Health Inspection Service Wildlife Services National Wildlife Research Center Invasive Species and Technology Development Research Program Analytical Chemistry Project	Invoice #: 09-005/5 Date: 05/15/2009 Page: 1 of 2

To: Chris Swenson
Pacific Islands Coastal Program
US Fish and Wildlife Service

Subject: Determination of Diphacinone in Whole Crabs

Method: 159A - Modified

Analysis Date: 04/13/09-04/14/09

AC Notebook Reference: AC 128 pp. 24-26

QC Notebook Reference: QC 29 p. 7

Analyst: Tom Primus

Sample Description: Crab samples arrived 01/23/09 and were logged into our sample tracking system. Samples arrived in Ziploc bags according to sample number with each crab individually wrapped in aluminum foil. Each tissue sample was homogenized in a SPEX liquid nitrogen freezer mill. Each homogenized sample was placed in a labeled bag, vacuum sealed and frozen (-20 °C) until analyzed.

Additional Comments: The mean MLOD was determined to be 0.200 ppm Diphacinone. Modifications to method 159A included the following. After evaporating the extraction solution, each sample residue was reconstituted with 2 mL chloroform and 3 mL hexanes. During filtering before cleanup, each sample tube was rinsed with 1 mL of both chloroform and hexanes. The solid phase extraction (SPE) cleanup procedure was completed with Phenomenex Strata X-AW 33 µm polymeric weak anion (200 mg) SPE columns conditioned with 1.0 mL chloroform and 1.5 mL hexanes. After loading each SPE column with the sample extract, each column was washed with a solution used to rinse the sample tube consisting of 1.0 mL chloroform and 1.5 mL hexanes. The analyte was eluted off each SPE column with 12 mL of 15 mM TBA in methanol and collected in a 10 mL screw top tube.

The mobile phase was replaced with 54% 5 mM TBA in Methanol : 46% Aqueous 5mM TBA / phosphate buffer solution with pH ~8.5 combined with a gradient. High performance liquid chromatograph used UV detection @ 325 nm for the analytical wavelength with 360 nm as the reference.

Analyst	Date	QC Specialist	Date	Reviewer	Date
Thom M. Primus	7/17/09		7/17/09	Thom M. Primus	7/17/09

Results:

Table 1. Diphacinone concentration in analyzed crab samples.

Sample Description	Date of Analysis	Lab ID	Diphacinone Conc. (ppm)
Baseline Crab - Control	04/13/09	S090107-08	<MLOD
Crab: Ama Site 1 Lehau JJH09-006	04/13/09	S090123-01	<MLOD
Crab: Ama Site 1 Lehau JJH09-009	04/13/09	S090123-02	<MLOD
Crab: Ama Site 1 Lehau JJH09-12	04/13/09	S090123-03	<MLOD
Crab: Ama Site 2 Lehau JJH09-005	04/13/09	S090123-04	<MLOD
Crab: Ama Site 2 Lehau JJH09-11	04/13/09	S090123-05	<MLOD
Crab: Ama Site 3 Lehau JJH09-004A	04/13/09	S090123-06	<MLOD
Crab: Ama Site 3 Lehau JJH09-004B	04/13/09	S090123-07	<MLOD
Crab: Ama Site 3 Lehau JJH09-10	04/13/09	S090123-08	<MLOD

MLOD = 0.200 ppm

Table 2. Quality Control Recovery for Diphacinone (Surrogate Corrected).

ID	Fortification Level (ppm)	% Recovery (surrogate corrected)
QC 1	Blank	-----
QC 2	Blank	-----
QC 3	0.520	161
QC 4	0.510	118
QC 5	2.06	126
QC 6	2.08	88.8
Mean		123 ± 30

Baseline Crab (Control) used for all QC samples (S090107-08)

Cc: Tom Primus
Doreen Griffin

Wildlife Services NWRC National Wildlife Research Center Analytical Services Report	United States Department of Agriculture Animal Plant Health Inspection Service Wildlife Services National Wildlife Research Center Invasive Species and Technology Development Research Program Analytical Chemistry Project	Invoice #: 09-005/1 Date: 02/12/2009 Page: 1 of 2

To: Chris Swenson
Pacific Islands Coastal Program
US Fish and Wildlife Service

Subject: Determination of Diphacinone in Fish Tissue

Method: 159A - Modified

Analysis Date: 02/09/09-02/11/09

AC Notebook Reference: AC 137 pp. 199-201

QC Notebook Reference: QC 26 p. 182-183

Analyst: Tom Primus

Sample Description: Fish samples arrived 01/23/09 and were logged into our sample tracking system. Samples arrived in Ziploc bags according to sample number with fish fillet individually wrapped in aluminum foil. Each tissue sample was homogenized in a SPEX liquid nitrogen freezer mill. Each homogenized sample was placed in a labeled bag, vacuum sealed and frozen (-20 °C) until analyzed.

Additional Comments: The mean MLOD was determined to be 0.015 ppm Diphacinone. Modifications to method 159A included the following. After evaporating the extraction solution, each sample residue was reconstituted with 2 mL chloroform and 3 mL hexanes. During filtering before cleanup, each sample tube was rinsed with 1 mL of both chloroform and hexanes. The solid phase extraction (SPE) cleanup procedure was completed with Phenomenex Strata X-AW 33 µm polymeric weak anion (200 mg) SPE columns conditioned with 1.0 mL chloroform and 1.5 mL hexanes. After loading each SPE column with the sample extract, each column was washed with a solution used to rinse the sample tube consisting of 1.0 mL chloroform and 1.5 mL hexanes. The analyte was eluted off each SPE column with 12 mL of 15 mM TBA in methanol and collected in a 10 mL screw top tube.

The mobile phase was replaced with 60% 5 mM TBA in Methanol : 40% Aqueous 5mM TBA : phosphate buffer solution with pH ~8.5. High performance liquid chromatograph used UV detection @ 325 nm for the analytical wavelength with 360 nm as the reference.

Analyst	Date	QA/QC Specialist	Date	Reviewer	Date
	2/10/09		2/17/09		2/16/09

Results:

Table 1 Diphacinone concentration in analyzed fish samples.

Sample Description	Date of Analysis	Lab ID	Diphacinone Conc. (ppm)
Baseline Fish - Control	02/09/09	S090107-07	<MLOD
Lehua Site 1 JJH 09-009 Hawkfish 12 Jan. 2009	02/09/09	S090123-20	<MLOD
Lehua Site 3 JJH 09-007 Hawkfish 12 Jan. 2009	02/09/09	S090123-28	<MLOD
Lehua Site 1 JJH 09-006 Hogfish 7 Jan. 2009	02/09/09	S090123-29	<MLOD
Lehua Site 1 JJH 09-006 Toau 7 Jan. 2009	02/09/09	S090123-30	<MLOD
Lehua Site 1 JJH 09-012 Wrasse 19 Jan. 2009	02/09/09	S090123-31	<MLOD
Lehua Site 2 JJH 09-008 Nenue 12 Jan. 2009	02/09/09	S090123-32	<MLOD
Lehua Site 3 JJH 09-004 Taape 7 Jan. 2009	02/09/09	S090123-33	<MLOD
Lehua Site 3 JJH 09-004 Hinalea 7 Jan. 2009	02/09/09	S090123-34	<MLOD
Lehua Site 3 JJH 09-007 Nenue 12 Jan. 2009	02/09/09	S090123-35	<MLOD
Lehua Site 1 JJH 09-006 Hawkfish 7 Jan. 2009	02/10/09	S090123-18	<MLOD
Lehua Site 1 JJH 09-009 Hawkfish 12 Jan. 2009	02/10/09	S090123-19	<MLOD
Lehua Site 2 JJH 09-005 Hawkfish 7 Jan. 2009	02/10/09	S090123-21	<MLOD
Lehua Site 2 JJH 09-005 Hawkfish 7 Jan. 2009	02/10/09	S090123-22	<MLOD
Lehua Site 2 JJH 09-011 Hawkfish 19 Jan. 2009	02/10/09	S090123-23	<MLOD
Lehua Site 2 JJH 09-011 Hawkfish 19 Jan. 2009	02/10/09	S090123-24	<MLOD
Lehua Site 3 JJH 09-004 Hawkfish 7 Jan. 2009	02/10/09	S090123-25	<MLOD
Lehua Site 3 JJH 09-004 Hawkfish 7 Jan. 2009	02/10/09	S090123-26	<MLOD
Lehua Site 3 JJH 09-007 Hawkfish 12 Jan. 2009	02/10/09	S090123-27	<MLOD

MLOD = 0.016 ppm on 02/09/09 and 0.014 ppm on 02/10/09

Table 2. Quality Control Recovery for Diphacinone (Surrogate Corrected).

ID	Fortification Level (ppm)	% Recovery (surrogate corrected)
QC 1	Blank	-----
QC 2	Blank	-----
QC 3	0.0979	96.3
QC 4	0.0998	93.8
QC 5	0.247	97.2
QC 6	0.254	96.3
QC 7	Blank	-----
QC 8	Blank	-----
QC 9	0.104	100
QC 10	0.0943	101
QC 11	0.242	90.3
QC 12	0.254	99.3
Mean		96.7 ± 3.6

Baseline Fish (Control) used for all QC samples (S090107-07B)

Cc: Tom Primus
Doreen Griffin

Wildlife Services NWRC National Wildlife Research Center Analytical Services Report	United States Department of Agriculture Animal Plant Health Inspection Service Wildlife Services National Wildlife Research Center Invasive Species and Technology Development Research Program Analytical Chemistry Project	Invoice #: 09-005/2 Date: 03/14/2009 Page: 1 of 2

To: Chris Swenson
Pacific Islands Coastal Program
US Fish and Wildlife Service

Subject: Determination of Diphacinone in Limpets and Whale Liver - Lehua

Method: 159A - Modified

Analysis Date: 03/04/09 – 03/05/09, 03/11/09

AC Notebook Reference: AC 137 pp. 171, 203

QC Notebook Reference: QC 26 pp. 71, 199

Analyst: Tom Primus

Sample Description: Limpet samples arrived 01/23/09 and were logged into our sample tracking system. Samples arrived in Ziploc bags according to sample number with limpet soft tissue wrapped in aluminum foil. Reference or baseline limpets (S090107-06) required soft tissue to be removed from shell before homogenization. Whale liver sample arrived 02/19/09 and was logged into our sample tracking system. Each tissue sample was homogenized in a SPEX liquid nitrogen freezer mill. Each homogenized sample was placed in a labeled bag, vacuum sealed and frozen (-30 °C) until analyzed.

Additional Comments: The MLOD was determined to be 0.052 ppm Diphacinone. Modifications to method 159A included the following. Methanol was used as the extraction solvent. After evaporating the extraction solution, each sample residue was reconstituted with 2 mL chloroform and 3 mL hexanes. During filtering before cleanup, each sample tube was rinsed with 1 mL of both chloroform and hexanes. The solid phase extraction (SPE) cleanup procedure was completed with Phenomenex Strata X-AW 33 µm polymeric weak anion (500 mg) SPE columns conditioned with 1.5 mL chloroform and 1.75 mL hexanes. After loading each SPE column with the sample extract, each column was washed with a solution used to rinse the sample tube consisting of 1.5 mL chloroform and 1.75 mL hexanes. The analyte was eluted off each SPE column with 12 mL of 15 mM TBA in methanol and collected in a 10 mL screw top tube.

For the whale liver sample acetonitrile was used as the extraction solvent and the sample was analyzed in triplicate. The MLOD was estimated to be 0.060 ppm Diphacinone. The quality control samples for this sample used quail liver as the control. Additionally the surrogate Chlorophacinone was added to the whale liver samples at 1.00 ppm and the recovery was 92.7±14.3%. Diphacinone and Chlorophacinone typically have similar chemical properties.

The mobile phase was replaced with 60% 5 mM TBA in Methanol : 40% Aqueous IPCA Solution with pH ~8.5. High performance liquid chromatograph used UV detection @ 325 nm for the analytical wavelength with 360 nm as the reference.

Analyst	Date	QC Specialist	Date	Reviewer	Date
	7/14/09		7/14/09		7/14/09

Results:

Table 1. Diphacinone concentration in analyzed limpet samples.

Sample Description	Lab ID	Diphacinone Conc. (ppm)
Opihi Limpets:Control Baseline Lehua n=3	S090107-06	<MLOD
Opihi Limpets: Site 1 Lehua JJH09-12	S090123-09	<MLOD
Opihi Limpets: Site 1 Lehua JJH09--006	S090123-10	<MLOD
Opihi Limpets: Site 1 Lehua JJH09--009	S090123-11	<MLOD
Opihi Limpets: Site 2 Lehua JJH09--005	S090123-12	<MLOD
Opihi Limpets: Site 2 Lehua JJH09--11	S090123-13	<MLOD
Opihi Limpets: Site 2 Lehua JJH09--008	S090123-14	<MLOD
Opihi Limpets: Site 3 Lehua JJH09--004	S090123-15	<MLOD
Opihi Limpets: Site 3 Lehua JJH09--007	S090123-16	<MLOD
Opihi Limpets: Site 3 Lehua JJH09--10	S090123-17	<MLOD

Table 2. Quality Control Recovery for Diphacinone.

ID	Fortification Level (ppm)	% Recovery
QC 1	Blank	------
QC 2	Blank	------
QC 3	0.189	88.3
QC 4	0.204	85.5
QC 5	0.979	97.8
QC 6	0.979	101
Mean		93.2 ± 7.4

Lehua Reference Limpets used for all QC samples (S090107-06)

Table 3. Diphacinone concentration in analyzed whale liver sample.

Sample Description	Lab ID	Diphacinone Conc. (ppm)
Whale liver	S090219-01	<MLOD

Cc: Tom Primus
Doreen Griffin

Wildlife Services NWRC National Wildlife Research Center Analytical Services Report	United States Department of Agriculture Animal Plant Health Inspection Service Wildlife Services National Wildlife Research Center Invasive Species and Technology Development Research Program Analytical Chemistry Project	Invoice #: 09-005/4 Date: 03/19/09 Page: 1 of 2

To: Chris Swenson
Pacific Islands Coastal Program
US Fish and Wildlife Service

Subject: Determination of Diphacinone in Seawater

Method: 158A - Modified

Analysis Date: 03/16/09

AC Notebook Reference: AC 137 pp. 204-205

QC Notebook Reference: QC 26 pp. 200

Analyst: Tom Primus

Sample Description: Water samples arrived 01/23/2009 and were logged into our sample tracking system. Water samples were in 250 mL screw top jars. Water samples were stored in a refrigerator at 4 °C until analyzed. All samples were analyzed with a modified version of method 158A. The method uses 150 mL of sample.

Additional Comments: The MLOD was 0.050 ppb Diphacinone and 0.024 ppb Chlorophacinone. Method 158A modifications included omitting step 3 (addition of salt to the sample to increase ionic strength of the sample) and replacing the mobile phase with 60% 5 mM TBA in Methanol : 40% Aqueous IPCA Solution with pH ~8.5. High performance liquid chromatograph used UV detection @ 325 nm for the analytical wavelength with 360 nm as the reference.

Analyst	Date	QC Specialist	Date	Reviewer	Date
T. M. Primus	7/16/09		7/16/09	T. M. Primus	7/16/09

Results:

Table 1. Diphacinone concentration in analyzed water samples.

Sample Description	Lab ID	Diphacinone Conc. (ppb)
Water: (Control) Baseline Lehau total = 3	S090107-05	<MLOD
Water: Site 1 Lehua JJH09--006	S090123-45	<MLOD
Water: Site 1 Lehua JJH09--009	S090123-46	<MLOD
Water: Site 1 Lehua JJH09--12	S090123-47	<MLOD
Water: Site 2 Lehua JJH09--009	S090123-48	<MLOD
Water: Site 2 Lehua JJH09--008	S090123-49	<MLOD
Water: Site 2 Lehua JJH09--11	S090123-50	<MLOD
Water: Site 3 Lehua JJH09--004	S090123-51	<MLOD
Water: Site 3 Lehua JJH09--007	S090123-52	<MLOD
Water: Site 3 Lehua JJH09--10	S090123-53	<MLOD

Table 2. Quality Control Recovery for Diphacinone (Surrogate Corrected).

ID	Fortification Level (ppb)	% Recovery (surrogate corrected)
QC 1	Blank	------
QC 2	0.554	111
QC 4	0.556	117
Avg.		114 ± 4.2

Lehua Baseline Sea Water used for all QC samples (S090107-05)

Cc:
Tom Primus
Doreen Griffin

Wildlife Services NWRC National Wildlife Research Center Analytical Services Report	United States Department of Agriculture Animal Plant Health Inspection Service Wildlife Services National Wildlife Research Center Invasive Species and Technology Development Research Program Analytical Chemistry Project	Invoice #: 09-005/3 Date: 04/29/2009 Page: 1 of 2

To: Chris Swenson
Pacific Islands Coastal Program
US Fish and Wildlife Service

Subject: Determination of Diphacinone in Soils - Lehua

Method: New Un-validated Method

Analysis Date: 03/24/09, 03/26/09, 03/27/09, 03/30/09, 04/03/09

AC Notebook Reference: RD 73 pp. 149 - 152

QC Notebook Reference: RD 73 pp. 150 – 151, QC 26 pp. 194-195

Analyst: Tom Primus

Sample Description: Limpet samples arrived 01/23/09 and were logged into our sample tracking system. Samples arrived in 250 mL glass jars wrapped in aluminum foil and sealed in labeled Ziploc bags. Soil samples S090123-37 and S090123-44 arrived with the sample jar cracked open, however the soil was contained within the sample bag. A 20 gram portion of each soil sample was homogenized using the SPEX liquid nitrogen mill. A 35 gram portion of reference or baseline soil (S090107-04) was homogenized. Each homogenized sample was placed in a labeled bag, vacuum sealed and frozen (-30 °C) until analyzed.

Additional Comments: The MLOD was determined to be 0.050 ppm Diphacinone and 0.071 ppm Chlorophacinone. The method was completed as follows. One gram aliquots of each sample were weighed into individual 100 mL round bottom flasks. Methanol with 1% concentrated formic acid was used as the extraction solvent. To each one gram aliquot of sample, 25 mL of extraction solvent was added. Each sample was refluxed for three hours at the boiling point of the extraction solvent. After cooling, the sample extracts were transferred to 25 mL tubes. Each round bottom flask was washed twice with 1 to 1.5 mL of methanol. After evaporating the extraction solution, each sample residue was reconstituted with 1.0 mL of mobile phase which consisted of 60:40 (5 mM TBA in Methanol : Aqueous IPCA Solution with pH ~8.5). Each sample was placed in an ultrasonic bath for ten minutes and the filtered through a 0.45um Teflon syringe filter into an HPLC vial. Additionally the surrogate Chlorophacinone was added to the soil samples at 0.500 ppm and the recovery was corrected with the surrogate. Diphacinone and Chlorophacinone typically have similar chemical properties.

The instrument parameters for the analysis are as follows:
Reversed phase ion-pairing high performance liquid chromatography (HPLC) utilized a C-18 Phenomenex Gemini column. The mobile phase consisted of 60:40 (5 mM TBA in Methanol : Aqueous IPCA Solution with pH ~8.5) with a gradient. The mobile phase was maintained at a temperature of 30 °C with a runtime of 30 minutes and a flow rate of 0.30 mL/min. The HPLC used UV detection @ 325 nm for the analytical wavelength with 360 nm as the reference.

Analyst	Date	QC Specialist	Date	Reviewer	Date
Thomas M. Primus	7/14/09	Donald Sullivan	7/15/09	Thomas M. Primus	7/14/09

Results:

Table 1. Diphacinone concentration in analyzed soil samples.

Sample Description	Date of Analysis	Lab ID	Diphacinone Conc. (ppm)
Soil: Control Baseline Lehua n=3	Run as QC 1 and QC 5	S090107-04	<MLOD
Soil: Site 1 Lehua JJH09--006	3/30/09, 4/03/09	S090123-36	<MLOD
Soil: Site 1 Lehua JJH09--009 Jar Broken	3/24/09, 3/27/09	S090123-37	<MLOD
Soil: Site 1 Lehua JJH09--12	3/26/09, 3/27/09	S090123-38	<MLOD
Soil: Site 2 Lehua JJH09--005	3/26/09, 3/27/09	S090123-39	<MLOD
Soil: Site 2 Lehua JJH09--008	3/26/09, 3/27/09	S090123-40	<MLOD
Soil: Site 2 Lehua JJH09--11	3/30/09, 4/03/09	S090123-41	<MLOD
Soil: Site 3 Lehua JJH09--004	3/30/09, 4/03/09	S090123-42	<MLOD
Soil: Site 3 Lehua JJH09--007	3/30/09, 4/03/09	S090123-43	<MLOD
Soil: Site 3 Lehua JJH09--10 Jar Broken	3/24/09, 3/27/09	S090123-44	<MLOD

Table 2. Quality Control Recovery for Diphacinone.

ID	Fortification Level (ppm)	Date of Analysis	% Recovery
QC 1	Blank	3/24/09, 3/27/09	------
QC 2	0.556	3/24/09, 3/27/09	108
QC 3	1.07	3/24/09, 3/27/09	113
QC 4	1.04	3/24/09, 3/27/09	113
QC 5	Blank	3/26/09, 3/27/09	------
QC 6	0.530	3/26/09, 3/27/09	112
QC 7	1.02	3/26/09, 3/27/09	107
QC 9	0.520	3/30/09, 4/03/09	141
QC 10	1.07	3/30/09, 4/03/09	127
Mean			117 ± 12.3

Lehua Reference Soil used for all QC samples (S090107-04 A, B, and C)

Cc: Tom Primus
Doreen Griffin